REMARKS

Claims 1, 2 and 10-13 have been amended to better conform to preferred U.S. patent practice. The claims have been amended to recite sequence identifiers, to remove broad and narrow ranges recited within the same claim and to remove improper multiple dependencies.

Applicants note that all sequences recited in the specification are represented in the Sequence Listing filed June 18, 2001. Contrary to the Examiner's assertion, Applicants do not believe a new Sequence Listing and CRF is required, and respectfully submit that the Preliminary amendments to the application could have been forestalled until the Application had been reviewed on the merits. However, to clarify the location of the recited sequences, Applicants have amended the specification to include sequence identifiers.

Support for these amendments to the claims and specification can be found throughout the application as filed. No new matter will be introduced into the application via these amendments to the specification and claims, accordingly.

In view of the foregoing, Applicants have responded to each of the concerns listed by the Examiner in the communication. An early and favorable action on the merits is

respectfully requested. Should any questions related to patentability arise, the Examiner is invited to telephone the undersigned to discuss the same.

Respectfully submitted,

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Enclosure: Appendix

APPENDIX
MARK UP VERSION SHOWING CHANGES MADE

The specification has been amended as indicated in the marked-up paragraphs below.

Replace the paragraph at page 8, lines 1-6, with:

Fig 1: Murine cDNA sequence encoding the CRAM-1 and CRAM-2 proteins. muCRAM-1 (SEQ ID NO.: 11) was subcloned in pcDNA3 vector and sequenced using Sp6 and T7 primers. muCRAM-2 (SEQ ID NO.: 12) was obtained as an IMAGE clone from EST library (Ac: AA690843 and W80145) and was sequenced in the pT7T3-Dpac vector using T7 and T3 primers.

Replace the paragraph at page 8, lines 18-19, with:

Fig 3: Alignment of CRAM-1 (SEQ ID NO.:13) and [CRAM-1] CRAM-2 (SEQ ID NO.:14) amino acid sequences. Gaps are indicated as dashed lines.

Replace the paragraph at page 8, lines 24-27, with:

Fig 6: Nucleic acid sequence of human CRAM-1 (upper panel, SEQ ID NO.: 17), complete amino acid sequence of human CRAM-1 (middle panel, SEQ ID NO.:15), and partial amino acid sequence of human CRAM-2 (SEQ ID NO.: 16).

Replace the paragraph at page 8, lines 28-38, with:

Fig 7: Targeted differential display using degenerated primers. (A): Nucleotide sequences of PCR primers encoding the sequences present in C2 Ig domains are shown. Two primers encode the same sequence due to the codons encoding Ser residue. The level of degeneracy is 4096 different forms for the primers encoding YRCXAS (SEQ ID NO.: 18)

and 2048 forms for the others. (B): The display of radioactive PCR products obtained with the YYCXAS1 (SEQ ID NO.: 20) primers is shown. The lanes correspond to the display of PCR product run on cDNA obtained from the t-end endothelial cell line (lane t-end), the B16 melanoma cell

Replace the paragraph at page 9, lines 18-26, with:

Fig 9: JAM (SEQ ID NO.: 21), CRAM-1 and CRAM-2 murine protein sequence alignment. The identical residues are shaded in gray. The overall identity is 36% between CRAM-2 and CRAM-1, 31% between JAM and CRAM-1 and 33% between JAM and CRAM-2; the respective homologies are 52%, 52% and 49%. The gaps are shown by dashes in the sequences. The canonical conserved residues (Cys and Trp) of the V and C2 domains are marked by an [asterix] asterisk.

Replace the paragraph at page 14, lines 1-24, with:

For 64 hours in 10 cm tissue culture dishes. As control, $5x10^5$ t.End.1 and $2.5x10^5$ B16 F10 cells were grown separately under the same conditions resulting in confluent monolayers after 64 hours. Total RNA was directly extracted in petri dishes with Trizol reagent following manufacturer's instructions (Gibco BRL, Paisley, Scotland). The cDNA was prepared from 5 μg of total RNA, employing oligo-dT (16-mer) primer and Superscript Reverse Transcriptase (Gibco BRL, Paisley, Scotland). The quality and the quantity of cDNA were checked by running 27 cycles of PCR on 1 μl of cDNA diluted 1:5, using primers specific for the housekeeping HPRT cDNA. Then the differential PCR was performed with the following degenerate primers: $^{5'}$TAYAGNTGYNNNGCYTCYAA$^{3'}$ (SEQ ID NO.: 1), $^{5'}$TAYCRGTGYNNNGCYTCYAA$^{3'}$ (SEQ ID NO.: 2), and

$^{5'}$TAYTAYTGYNNNGCYTCYAA$^{3'}$ (SEQ ID NO.: 3), encoding for the most frequent amino acid sequences encountered in C2 domains: YRCXAS (SEQ ID NO.:18), YQCXAS (SEQ ID NO.:19), and YYCXAS (SEQ ID NO.:20). The PCR conditions consisted [in] of using 2 µl of diluted cDNA; 2.5 µl of 10X Goldstar PCR buffer; 2 µl of $MgCl_2$; 2 µl of degenerated primers [0,3] 0.3 mM; 0.5 µl of dNTP 0.1mM; 0.1 µl of αp^{33} dATP 10 mCi/ml (Amersham Pharmacia Biotech, Dubendorf, Switzerland); 15.65 µl H_2O; 0.25 µl Goldstar Taq polymerase (Eurogentech, Seraing, Belgium).

Replace the paragraph at page 15, lines 24-38 with:

The three primers used were designed based on the EST sequences as follows: [5'] $^{5'}$- GAGGTACTTGCATGTGCT-$^{3'}$ [3'] (SEQ ID NO.: 4) for synthesis of the first strand, [5'] $^{5'}$- CGACAGGTGTCAGATAACA$^{3'}$ [3'] (SEQ ID NO.: 5) and [5'] $^{5'}$- CACCCTCCTCACTCGT$^{3'}$ [3'] (SEQ ID NO.: 6) for the two nested PCRs. The 5' RACE-PCR product was cloned into pGem-T [Vector] vector. To obtain the full length coding sequence for CRAM-1, the cloned 5'RACE-PCR product and the EST (accession No. AA726206) were digested with HpaI and NotI restriction enzymes and ligated into pGem-t vector. Cloning of full length CRAM-2 was based on the same strategy of sequence comparison and 5'RACE technique. The full-length cDNA encoding CRAM-2 was finally obtained from ESTs accession numbers: AA690843 and W80145. These two clones differ by the length of the 3' untranslated region.

Replace the paragraph at page 16, lines 26-35, with:

Semi-quantitative PCR reaction or [northern] Northern blotting were used to determine relative amount of transcript in the various conditions. For the detection of the JAM-2 transcript, the $^{5'}$-GACTCACAGACAAGTGAC-$^{3'}$ (SEQ ID NO.: 7) and

5'-CACCTCCTCACTCGT-3' primer pair was used, giving a 750 bp amplification product. As internal control, the following primers specific for Hprt cDNA were used to amplify a 350/bp long fragment: 5'-GTTGGATACAGGCCAGACTTTGTTG-3' (SEQ ID NO.:9) and 5'-GAGGGTAGGCTGGCCTATAGGCT-3' (SEQ ID NO.:10).

Replace the paragraph at page 23, lines 22-36, with:

The putative structure of the murine CRAM-1 protein is shown in Fig 8B and consists of an extracellular region with a variable heavy chain and a constant type 2 like immunoglobulin domain (Pfam, The Sanger Centre and Blast) with two potential N-linked glycosylation sites (aa 104 and 192). The hydrophobicity analysis (tmpred, ISREC) predicted a transmembrane region between positions 242-260. The postulated cytoplasmic domain consisted of 49 amino acids and contained a number of highly conserved Ser/Thr and Tyr phosphorylation sites (Fig 8A, residues in italic). The search of known patterns with the Prosite program identified the motifs SSk/SYK as protein kinase C, SKQD (amino acids 296-299 of SEQ ID NO.: 13)/TSEE (amino acids 275-278 of SEQ ID NO.: 13) as CK2 and KQDGESY(amino acids 276-281 of SEQ ID NO.: 13) /KHDGVNY(amino acids 287-293 of SEQ ID NO.: 13) as Tyrosine kinase phosphorylation signatures.

IN THE CLAIMS:

The claims have been amended as indicated below.

1. (Amended) An isoalted polypeptide [Polypeptide in isolated form] belonging to a subfamily of the human Immunoglobulin Superfamily, which polypeptide shows at least 70% sequence homology with the amino acid sequence of the murine Confluency Regulated

Adhesion [Molecules] Molecule 1 (CRAM-1, SEQ ID NO: 13) or CRAM-2 (SEQ ID NO.: 14) [(CRAM-1 or CRAM-2) as depicted in Fig 3 upper and lower row, respectively].

2. (Amended) The polypeptide [Polypeptide] as claimed in claim 1 comprising an amino acid sequence that is [at least] 70% to essentially 100% [, preferably at least 80%, more preferably at least 90%, most preferably essentially 100%] homologous to the amino acid sequence of human CRAM-1 (SEQ ID NO.: 15) [as depicted in Fig 6].

11. (Amended) A polypeptide [Peptide] having at least part of the amino acid sequence of the polypeptide as claimed in claims 1 [and] or 2 for use in the treatment of inflammation reactions.

12. (Amended) The polypeptide [Peptide] as claimed in claim 11, wherein the at least part of the amino acid sequence comprises at least one of the extracellular domains VC2, and [/or] the membrane proximal cytoplasmic sequence [:] defined by amino acids 266-272 of SEQ ID NO.: 13 or amino acids 261-267 of SEQ ID NO.: 14 [A-[Y,Q]-[R,S]-[R,K]-G-[C,Y]-F].

13. The polypeptide [(Poly)peptides] as claimed in claims 1, or 2 [, 11, and 12] in soluble form for use in modulating vascular permeability.